

Protein Footprinting of RNase Peptide

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RNase S-peptide forms a catalytically active complex with the RNase S-protein. The oxidative products obtained from the S-peptide present as a monomeric solution and as a complex with the S-protein will be characterized. Since the hydroxyl radical generated by radiolysis reacts with the solvent accessible surface of protein-DNA complexes, the solvent accessible surfaces of the S-peptide should also be subject to oxidation. Residue sidechains whose solvent accessible surface is buried on complex formation should be protected from oxidation in the complex. The experimental protocol required to normalize the observed mass spectral data will be developed. The availability of a crystal structure permits the pattern of residue oxidation to be carefully analyzed in terms of solvent accessibility. If differences in the accessibility of oxidatively modified residues are identified, the time course of the interaction of the S-peptide with the S-protein will be examined by stopped-flow radiolysis. Preliminary data already show the protection of the peptide methionine and phenylalanine when bound to protein. Time-resolved studies will be used to establish the mechanisms of binding.